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The Role of Osteogenic Cells in the Pathophysiology of Paget's Disease

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INTRODUCTION

FIRST RECOGNIZED and described in detail by Sir James Paget in 1877,[1] Osteitis deformans (Paget's disease of bone) is a focal disease that can be monostotic or polyostotic. Pagetic lesions are characterized by three somewhat diffuse phases, recognized by 1) an initial burst of osteoclastic activity, 2) a highly increased level of bone turnover leading to deposition of structurally abnormal bone, and 3) the eventual cessation of bone cell activity, with bone formation outweighing bone resorption. These processes result in a unique structure that is both porous and sclerotic and composed of thick but mechanically unsound bone trabeculae. However, the etiology and pathogenesis of Paget's disease of bone has not been clarified. A viral etiology was first postulated by identification of intranuclear paracrystalline inclusions reminiscent of paramyxovirus-related particles in osteoclasts.[2] Similar inclusions have been demonstrated in osteoclasts and in nonosteoclastic giant cells in other diseases of bone (including pyknodysostosis, giant cell tumors, and primary oxalosis) not related to Paget's disease.[3–6] However, evidence for the presence of viruses in pagetic bone has also been repeatedly obtained using immunologic or molecular approaches. Different paramyxoviruses, including measles, Rous sarcoma virus (RSV), and canine distemper virus, have been implicated.[7–10] Results have not been uniform, however, and negative data have also been obtained by analyzing pagetic bone using reverse transcriptase polymerase chain reaction (RT-PCR) techniques.[11,12] While it is clear that osteoclastic activity is abnormal in Paget's disease, the purpose of this review is to highlight the potential role of osteogenic cells in the pathophysiology of the disease. We feel that a reexamination of pagetic osteogenic cells is warranted based on recent and rediscovered findings in osteogenic cell biology that provide new insight into the function of the bone/bone marrow organ in health and in disease.

THE CELLULAR BASIS OF PAGET'S DISEASE

Irrespective of the role that one or more (paramyxo)virus(es) may play in causing Paget's disease, the relative role of different cell types within the bone/bone marrow organ in establishing the pagetic lesion and promoting its progression or arrest is also unclear. The occurrence of lytic lesions in pagetic bone in the early phases of the disease, the consistent evidence of obvious structural and ultrastructural changes in the pagetic osteoclasts, and the favorable response of the disease to antiresorptive agents[13,14] represent the main tenets that support the widely held belief that Paget's disease is essentially a disease of bone resorption. Caution should be exerted, however, in separating out as clearly as possible the unquestionable role that bone resorption has in the development of pagetic lesions from the identification of the essential cellular target of whatever critical pathogenetic factor (viral or otherwise) underlies the development of the disease. While the demonstration of viral infection of osteoclasts as the causative mechanism is not definitive, it is clear that osteoclasts are abnormal[15] in Paget's disease. As discussed below, bone formation is also severely abnormal in Paget's disease, and different evidence points to an involvement of cells of the osteogenic lineage that can hardly be dismissed as merely secondary to the increased rate of bone remodeling.[16] Current acquisitions on the biology of osteoclastogenesis, however, would rather argue that even some aspects of the increased resorption observed in pagetic bone (namely, the recruitment and differentiation of osteoclasts),[17,18] may be mediated by cells in the stromal/osteoblastic lineage.

OSTEOGENIC CELLS IN PAGET'S DISEASE

In view of the wide prevalence of Paget's disease, and of the wider use of "osteoblastic" models of various nature and identity in bone biology, the paucity of information on

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the biology of osteogenic cells in Paget's disease is perhaps disconcerting. Viral nucleocapsid proteins and other antigens have been detected in osteoblastic cells of pagetic bone in vivo and in cell cultures derived from pagetic bone by in situ hybridization and immunohistochemistry,[7–9,19–21] which in itself should have prompted a more thorough investigation of the pagetic osteoblast. In addition, evidence indicates that cells in the osteoblastic lineage are active participants in the pathogenesis of the pagetic bone lesion. First, the bone deposited within the lesion (which represents the lasting footprint of osteoblastic cell function) is structurally abnormal. Second, cellular changes observed in the developing and established pagetic lesion clearly point to osteoblastic involvement. Third, regulatory events and changes in the cytokine milieu that are thought to be altered in Paget's disease are at least in part mediated by, or influenced by, the biology of stromal cells, which include osteogenic cells. Fourth, and often overlooked, not only do bone tumors of osteogenic or stromal lineage develop in Paget's disease,[22] but this may be due to the same genetic alterations associated with familial Paget's disease.

The histopathology of Paget's disease

We have reviewed, from our files, a series of seven iliac crest biopsies taken from patients known to be affected by Paget's disease but with no overt evidence of iliac bone involvement ($n = 4$), or patients in which Paget's disease was first indicated by iliac crest biopsy findings and later confirmed by proper general clinical evaluation ($n = 3$) as well as by subsequent detection of typical virus-like nuclear inclusions in osteoclasts. Microscopic evidence for pagetic changes in the absence of overt clinical involvement of a bone site can be taken as indicative of an early pagetic lesion. This principle applies to a number of bone diseases such as hyperparathyroidism, where bone biopsy can disclose microscopic changes in the iliac bone in the absence of macroscopic and X-ray evidence of macroscopic "osteitis fibrosa." In a strictly histological sense, we regard as early those changes that do not feature extensive fibrosis and subversion of the marrow and bone structure. Early pagetic lesions are localized at endosteal regions of trabecular bone and within the adjacent bone marrow. In proximity to sites of trabecular bone resorption, vascular dilations and sometimes hyperplasia of hematopoietic cells are demonstrated (Fig. 1).[23] During the active phase of the disease, changes detected in the bone marrow are even more obvious. These include the loss of hematopoietic activity and cells, and the development of an abnormal stromal tissue noted as "fibrosis," which comes to fill entirely the intertrabecular spaces. Interestingly, as is often the case with other bone diseases, similar changes are detectable on a smaller scale in biopsies that are taken from bones that are not known to be clinically (i.e., grossly) involved. In most cases, these changes do not reflect secondary hyperparathyroidism, and truly represent a microscopic (and perhaps not necessarily progressive) stage in the establishment of pagetic lesions. In iliac crest biopsies showing early pagetic involvement, the fibrosis that extensively occupies the marrow in a full-blown

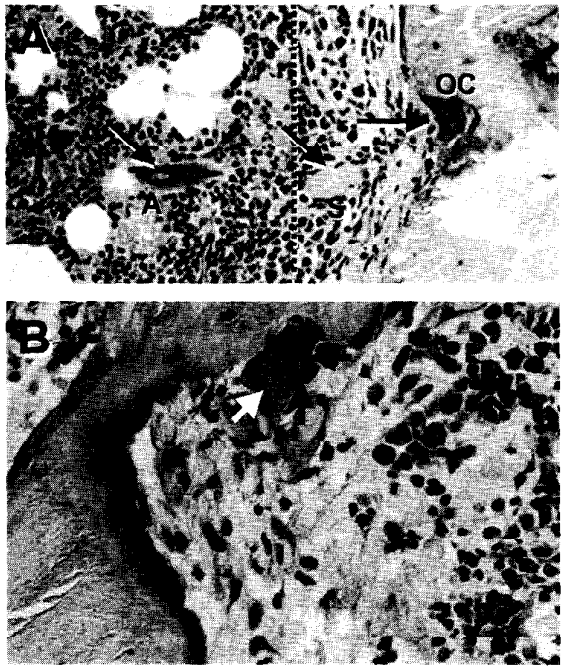

FIG. 1. Early pagetic lesions as revealed by iliac crest biopsy taken from a grossly uninvolved iliac bone demonstrating microscopic pagetic changes. (A) A localized loss of hematopoietic cells in the peritrabecular endosteal region is noted, which contrasts with the hyperplastic marrow further away from the bone surface. A dashed line marks the boundaries between the two regions. Note that a cutting cone is forming over the trabecular surface, where an osteoclast is found. The osteoclast (OC) is aligned along an axis conjoining a small artery (A) and a sinusoidal branch (S) thereof, suggesting the activation of osteoclastic resorption in conjunction with a vascular/circulatory change. (B) Detail of a peritrabecular region. The osteoclast contains nuclear inclusions (black arrows) that can be shown by electron microscopy to represent typical virus-like structures, and a large cytoplasmic inclusion (white arrow). Note the loose "fibrosis" adjacent to the active osteoblastic surface, the absence of hematopoiesis, and the prominence of large sinusoidal blood vessels.

pagetic lesion is spatially restricted to endosteal regions. Within the abnormal, "fibrotic" tissue, hematopoietic cells are depleted and marrow sinusoids are markedly dilated. The associated endosteal surface is undergoing active remodeling, and abnormal osteoclasts bearing nuclear inclusions typical of Paget's disease are readily detected. When low temperature processed, glycol methacrylate embedded bone biopsies are used in enzyme cytochemical assays, the "fibrotic" tissue is revealed to be composed of an excess of elongated, delicately branched stromal cells that express high levels of alkaline phosphatase and thus resemble the preosteogenic stromal cells found in the normal bone marrow (also known as Westen–Bainton cells, or reticular cells) (Fig. 2).[24] Of note, these cells are a structural component of the marrow sinusoid walls in the normal bone marrow,

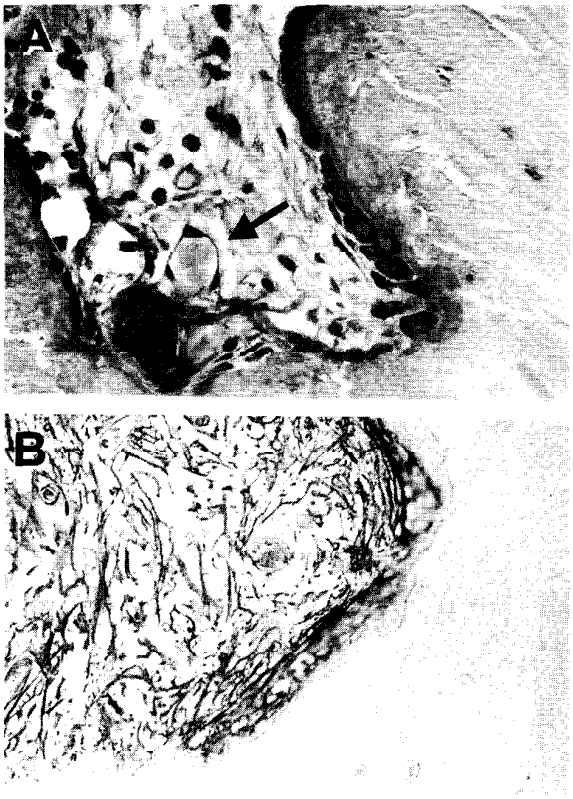

FIG. 2. Demonstration that the peritrabecular "fibrosis" is comprised of osteogenic (alkaline phosphatase-positive) stromal cells. (A) Shows a Giemsa-stained section in which the peritrabecular region is devoid of hematopoiesis and appears loosely fibrotic with prominent blood vessels (arrow). (B) Shows a similar field in a section reacted for alkaline phosphatase. Note the crowding of elongated, alkaline phosphatase-positive stromal cells.

which are thought to participate in regulating caliber and blood flow within marrow blood vessels.[24] Thus, early and full-blown pagetic lesions involve distinct changes in the number, arrangement, and function (as indicated by the loss of the ability to maintain a normal hematopoietic environment) of stromal cells of the endosteal/medullary tissue. Changes in this cell population are common in other bone diseases in which involvement of the osteoblastic lineage is primary, such as hyperparathyroidism[25,26] and fibrous dysplasia,[27] but have not been previously recognized in Paget's disease since techniques commonly used for histology and histomorphometry do not allow for their detection.

An excess of marrow preosteoblastic cells at the site of a developing pagetic lesion is in keeping with earlier data on static and dynamic histomorphometry in Paget's disease. These data showed that not only is bone formation activity and rate increased in Paget's disease, but there is also an increased "birthrate" of osteoblasts.[28] The quality of the newly formed bone is woven in nature, and in some instances the bone can become hypermineralized. However, this is not always the case, as established pagetic lesions can become entirely or nearly entirely lamellar in structure, even though the occurrence of an unusually high number of turnover events remains clearly delineated by the extensive system of reversal or cement lines, leading to the characteristic pattern of Schmorl's mosaic.[29] The sum total of the stromal/osteoblastic abnormalities is an increase in bone mass, noted by increased density and thickness of trabecular structures, but with compromised mechanical integrity due to poor architectural organization, and perhaps due to changes in the material properties of the mineralized matrix as well.

Abnormal bone matrix chemistry in Paget's disease

A remarkable feature of pagetic lesions is the deposition of primarily woven versus lamellar bone, reminiscent of fetal development rather than adult bone turnover. The biochemical differences between woven and lamellar bone have not been well characterized. Although one of the most apparent changes in pagetic lesions is the abnormal deposition of extracellular matrix, there is a notable paucity of studies that address this issue. It has not been determined if the increase in bone matrix production is due to an increased biosynthetic output on a per cell basis, or simply a consequence of increased stromal/osteoblastic cell number. Either way, it has been reported that collagen fibrils of pagetic bone are of nonuniform size[30] compared with normal age-matched controls, which may also relate to the woven nature of the bone. In addition, decreased β-isomerization of the C-terminal propeptide has also been detected. It is not known what role β-isomerization plays in collagen deposition; however, quantification of the level of this isoform has been shown to be a useful biochemical marker in this disease.[31]

Noncollagenous proteins (NCP) play an important role in the organization and mineralization of bone matrix[32] and promote distinct cell–matrix interactions necessary for normal remodeling. Significant differences were observed in the distribution of osteopontin, and to a lesser extent, osteonectin, osteocalcin, and decorin, whereas no changes were noted for biglycan. The changes noted were associated at specific sites within the bone (the Haversian system in cortical bone, and in subperiosteal bone).[33] There have been a number of analyses performed on the changes in proteins secreted by osteoblastic cultures derived from pagetic bone. In one study, a number of up-regulated and down-regulated proteins secreted by pagetic osteoblastic cultures were visualized using 2D-protein mapping. However, these proteins have remained unidentified to date.[34] In another study, it was determined that human osteoblastic cultures secrete different immunoreactive forms of osteocalcin. Using the same assays, altered levels of the different immunoreactive forms of osteocalcin were detected in the serum of Paget's patients, suggesting an altered biosynthetic activity of pagetic osteoblasts.[35] Consequently, not only is the structure of pagetic bone abnormal, but the composition of the bone appears also to be altered, a factor that may impact on the mechanical properties.

Paracrine/autocrine factors, cell–cell interaction, and stromal/osteoblastic cells

Cells in the bone microenvironment that are in close association with osteogenic cells include the cells of the vasculature (pericytes, smooth muscle cells, endothelial cells), hematopoietic cells from which osteoclasts are formed, and the stromal cells themselves, including hematopoiesis supportive stromal cells and adipocytes. Factors that are produced and secreted by any of these cells types can influence osteoblastic metabolism, and changes induced in these intimately associated cell types by the disease process may result in abnormal stromal/osteoblastic behavior. As an example, one of the hallmark features of active pagetic lesions is the hypervascularity of the site. Recently, it has been determined that an endothelial cell product, ET-1, is elevated in the serum of pagetic patients.[39] Due to the fact that ET-1 has been reported to decrease osteoclastic bone resorption and increase osteoblastic cell proliferation, this raises the intriguing possibility that high levels of ET-1, produced by the increased number of endothelial cells, contributes to the imbalance of bone turnover, favoring bone formation.

Considerably more information is available concerning the influence of cytokines and growth factors that are secreted by osteoclasts on osteoblastic metabolism. Secretion of some of these factors by pagetic osteoclasts, such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), epidermal growth factor (EGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor beta (TGF-β) has been reported to be increased.[15,37–43] Several of these factors, such as PDGF, EGF, and TGF-β, have been shown to influence the proliferation and differentiation of bone marrow stromal cells (reviewed in[44]). Consequently, overabundance of these factors, either on a per osteoclast basis or due to the increase in the total number of osteoclasts, may contribute to the altered metabolism of the bone marrow stroma and subsequent differentiation into the osteoblastic phenotype. In addition, stromal/osteoblastic cells themselves produce many of the same cytokines and growth factors, which may further exacerbate the dysregulation of the cells within a lesion. However, reports characterizing several of these cytokines (IL-6, IL-1β, IL-1α, TNF-α) synthesized by pagetic osteoblastic cells failed to demonstrate any significant increase over levels synthesized by normal cells[41] with the possible exceptions of IL-6 and TNF-α.[37,43]

In addition to the above mentioned cytokines that are known to be involved in the regulation of bone resorption, other factors that are responsible for osteoclast formation as directed by stromal/osteoblastic cells have recently been more clearly delineated. Osteoclast formation in cocultures of stromal/osteoblastic cells and hematopoietic cells that contain osteoclast precursors is a process that is dependent on cell–cell interactions, a fact that led to the suggestion of stromal/osteoblastic synthesis of a putative osteoclast differentiation factor (ODF).[17] It has recently been demonstrated that ODF is TNF related activation-induced cytokine/receptor activator of NF-Kappa B ligand (TRANCE/RANKL), a member of the membrane-associated TNF ligand family. Differentiation of osteoclasts is directly regulated by binding of stromal cell-associated RANKL to RANK, presumably expressed by osteoclast precursors. Furthermore, the synthesis of RANKL is up-regulated by factors that increase bone resorption, and it binds to osteoclastogenesis inhibitory factor (OIF),[18] also known as osteoprotegerin.[45,46] The binding of osteoprotegerin by stromal/osteoblastic cells further illustrates how they are essential in the control of bone resorption. Both positive (by RANKL) and negative (by osteoprotegerin) regulation of osteoclast differentiation is linked via the modulation of a single molecular pathway to the interaction of stromal osteogenic cells with osteoclast precursors. Consequently, it is of interest to note that CD34-negative (stromal/osteoblastic cells) derived from pagetic bone initiate the formation of abnormal osteoclasts from normal hematopoietic cells in vitro,[47] lending strong support to the concept of stromal/osteoblastic cells playing a primary role in Paget's disease. The potential interactions of cells in the stromal/osteogenic lineage and osteoclastic cells, mediated via expression of factors, is shown in Fig. 3.

Paget's sarcoma and its tumor suppressor gene

The occurrence of sarcomas against the background of Paget's disease has been recognized as long as the disease itself, since Sir James Paget's first case also developed a sarcoma. Sarcomas arising in Paget's disease include osteogenic sarcoma as a major contributor, but also sarcomas in which a prevalent chondrogenic or fibrogenic differentiation, or a combination of these three factors, are detected.[22] It should not be overlooked that both osteogenic sarcoma proper (that is, a malignant mesenchymal tumor featuring neoplastic bone formation by the tumor cells) and the other types of Paget's sarcoma obviously point to a transforming event that takes place within cells of the stromal cell lineage, which include osteogenic cells. From the pioneering work of Friedenstein,[48] Owen and coworkers,[49] and more recent studies,[44] it is now well recognized that stromal cells, in addition to forming bone, can also form cartilage and other connective tissues. It is entirely conceivable that a secondary, increased growth rate of cells in this lineage may simply make them more prone to mutational events ultimately leading to transformation. However, the occurrence of stromal/osteogenic tumors, and those with chondrogenic and fibrogenic phenotypes within Paget's disease, could be seen as the most compelling evidence for a direct involvement of the stromal/osteoblastic lineage in the pathogenesis of the disease. It has long been noted that in some families there is clearly a genetically inherited trait that is associated with development of Paget's disease. Recent studies of several large kindreds have identified 18q21-22 as one possible locus.[50–52] However, not all familial Paget's can be mapped to this locus, indicating that there are other potential genes yet to be identified. An histocompatability locus antigen (HLA) site, 6p21.3, has also been associated with certain families.[53,54] Recently, a putative tumor suppressor gene locus associated with osteosarcoma has been mapped to the very same

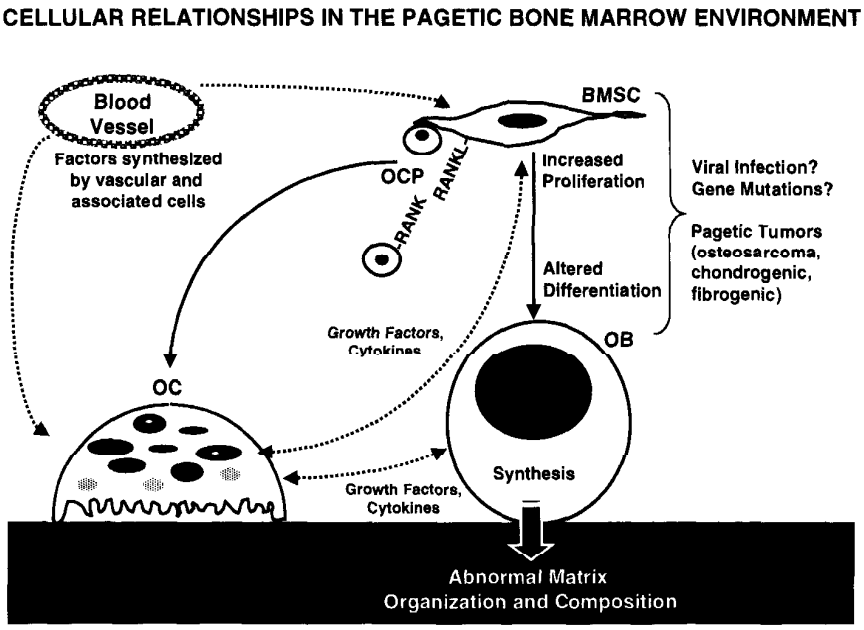

FIG. 3. Cellular relationships in the pagetic bone marrow environment. within the bone marrow microenvironment, all of the cell types present influence each other via cell–cell interactions and production of growth factors and cytokines. Given the hypervascular nature of early pagetic lesions, factors produced by the vascular and associated cells may influence activity of not only stromal/osteoblastic cells (BMSC and OB), but also osteoclastic cells (OC). In early stages of lesion formation, there is an increase in proliferation of BMSCs. Furthermore, these cells influence the formation of osteoclasts from osteoclastic precursors (OCP) via interactions that are mediated in part by RANKL (present on stromal cells) and RANK (on the surface of osteoclastic precursors), and consequently play an active role in regulating bone resorption. The result of the altered activity of stromal/osteoblastic cells (induced by production of factors by abnormal osteoclasts or other cells in the marrow, viral infection or genetic mutation) is the production of an extracellular matrix with abnormal organization and composition. Furthermore, pagetic lesions frequently undergo transformation into osteosarcomas, and tumors with chondrogenic and fibrogenic nature, further indicating a derangement in stromal/osteoblastic cells.

region of chromosome 18 as the locus associated with Paget's disease. Osteosarcomas occurring in Paget's disease have been found to display a loss of heterozygosity in this region,[55] which may be seen as an important piece of evidence in support of the view that cells in the osteoblastic lineage are primarily involved in Paget's disease.

MODEL SYSTEMS FOR FUTURE STUDIES

In spite of the fact that there has been some progress in delineating the potential pathophysiological process leading to the formation of pagetic lesions, there is much yet to be determined, especially in regard to the derangement of stromal/osteoblastic metabolism. Consequently, there is a need for the continued development of 1) in vitro model systems that ask specific questions with respect to the progression of osteogenic cells through different stages of maturation and changes in their metabolic activity, and 2) in vivo and animal models. Such studies are necessary for refinement of current therapies (e.g., treatment with various bisphosphonates aimed at particular osteoclastic functions, and at particular doses). Furthermore, it is clear that there is a need for the development of new therapies, considering the fact that 40% of patients with Paget's disease do not respond to treatment, even after aggressive bisphosphonate administration.

In vitro analysis of pagetic osteogenic cells

There are a large number of procedures that can be utilized to establish osteoblastic cells in vitro, including the use of bone marrow stromal cells,[56] release of osteoblastic cells from bone specimens by collagenase treatment, explants of fragments of trabecular bone with or without prior treatment with collagenase, etc. (reviewed in[57]). Several of these methods have been previously applied to the study of osteoblastic cells derived from pagetic bone.[19,34,35,37] If parameters of the starting material (sex, age, and site-matched normal donor) and cell culture parameters (initial plating density, length of time in culture, final density) are tightly controlled, any of these populations may be utilized to study changes in osteoblastic metabolism as a result of the disease process. However, it must be recognized that such cultures are inherently heterogeneous in that they represent osteoblastic cells at various stages of maturation, as is found during bone formation in vivo. In addition, osteoblastic cultures are removed from the influence of other cell types that may be actively involved in directing the meta

bolic activity of osteogenic cells. Furthermore, it must be recognized that while the deposition of mineralized matrix can be initiated in vitro, the structure and spatial relationships of tissues comprised in bone as an organ (the bone/marrow organ) cannot be recapitulated in vitro.

In spite of these limitations, these types of cultures can be utilized to measure certain parameters, to a certain extent, such as the determination of the number of osteogenic cells in marrow (colony forming efficiency of clonogenic stromal cells), rate of proliferation, expression of early markers of osteoblastic differentiation (alkaline phosphatase activity, and certain bone matrix proteins), and late markers of osteoblastic maturation (osteopontin, bone sialoprotein, osteocalcin). Furthermore, the effect of potential causative agents (either viral or biochemical in nature) on osteoblastic metabolism can be analyzed and compared to changes in osteoblastic function as delineated by in vivo analysis. In addition to currently standard analyses, there is a series of new technologies that, if utilized properly, have the potential to shed new light on the metabolic changes associated with Paget's disease. These include the analysis of mRNA populations (or cDNAs derived from them) via subtractive hybridization[58] and microarrays.[59] While still in their infancy, these techniques promise to be quite useful in delineating changes in gene expression patterns in various disease states.

In vivo models

If appropriately modeled, in vitro cultures can provide a method for answering specific mechanistic questions, however, in vivo models are a necessary adjunct, especially in regard to understanding the processes by which bone as an organ (which includes hematopoietic marrow) is formed. Due to the interrelationship and interactions of bone-forming cells with other tissues, such as the vasculature and hematopoietic cells,[24] true bone formation can only be studied in vivo.[60] The development of transgenic animals that either overexpress or are null (knockout) for a protein of interest offers a potentially informative avenue to examine a pathogenetic process. For example, transgenic animals that overexpress *tax*, exhibit pagetic changes in the skeleton[61] and those that overexpress c-*fos*, develop osteosarcomas.[62] Once genes associated with Paget's disease are identified, development of transgenic animals with deletions or mutations of these genes should provide valuable models for further study on the pathogenesis of this disease.

In addition, a novel model system can be utilized for the study of pathophysiology, but more importantly, to determine the relative role of different cell types in the pathogenesis of Paget's disease. This model is based on the fact that bone marrow stromal cells have the ability to regenerate a complete bone/bone marrow organ when transplanted subcutaneously into immunocompromised mice in association with appropriate carriers.[63] Consequently, any alteration of bone marrow stromal cell metabolism, either by genetic mutation or induced by viral infection, or any other etiology, would alter their ability to establish such a bone/bone marrow organ. The proof of this principle has recently been established in the study of fibrous dysplasia of bone, a disease that arises from an activating mutation of GNAS1 gene which codes for Gs alpha, and leads to overproduction of cAMP. When populations of bone marrow stromal cells containing the mutation were used in the in vivo transplantation system, a fibrous dysplastic ossicle was generated that recapitulated the changes in bone as an organ that were observed in patient-derived fibrous dysplastic tissue.[64] In the context of Paget's disease, this in vivo transplantation system could be utilized in two different ways. First, the nature of an ossicle generated by bone marrow stromal cells derived from pagetic tissue can be evaluated. Second, an ossicle can be generated with normal bone marrow stromal cells and, following appropriate treatment, human pagetic hematopoietic marrow cells can be introduced into the mouse to determine changes in bone turnover in the ossicle. Thus, the relative importance of stromal/osteogenic cells versus hematopoietic osteoclast precursors in the development of abnormal remodeling units in bone could be directly dissected out.

CONCLUSION

Although Paget's disease has been a well identified entity for over 100 years, the causative agent (whether it be viral infection or genetic mutation, or a combination of the two) is not yet known. Since the first observable change is an increase in bone resorption (determined by radiography), the osteoclast has been a primary focus in studying the pathophysiology of the disease. However, given current understanding that stromal/osteoblastic cells direct osteoclast formation, it cannot be ruled out that stromal/osteoblastic cells initiate this disease. Clearly, osteoblastic metabolism is either indirectly or directly altered in the formation of pagetic lesions as characterized by increased proliferation of stromal/osteoblastic cells, and their subsequent differentiation that generates a bone of abnormal character that may also be of low quality from a biomaterials point of view. Future studies utilizing new molecular technologies, as well as generation of appropriate in vitro and in vivo models, are necessary in order to gain a better understanding of the pathophysiology of this disease and to develop more effective therapies.

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